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The Effect of Ganglion-Blocking Agents on Survival of Rats Subjected to Acute Thermal Injury

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THE EFFECT OF GANGLION-BLOCKING AGENTS ON SURVIVAL OF RATS SUBJECTED TO ACUTE THERMAL INJURY

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THE EFFECT OF GANGLION-BLOCKING AGENTS ON SURVIVAL OF RATS SUBJECTED TO ACUTE THERMAL INJURY

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THE survival-promoting quality of adequate fluid and electrolyte replacement in burned man and experimentally burned animals has recently received thorough verification.[5, 15-17] On the other hand, a recent renewed attempt to improve survival rates by means of a pharmacologic agent[11] has not been confirmed.[14] The lowered mortality in humans suffering from extensive burns, as indicated by a recent study,[18] can be attributed in large part to the rapidity and adequacy with which fluid therapy has followed the initial burn.

The part played by the nervous system in producing the picture of shock, although accepted as important, is far from having been elucidated. Nevertheless, a partial insight into the mechanism relating the nervous system to certain hemodynamic phenomena has been recently provided by evidence indicating that adequate block of efferent autonomic impulses may, if properly controlled, reduce the blood loss at the operation site and thus reduce the frequency of shock associated with traumatic procedures.[6] This phenomenon, known as controlled hypotension, in contrast to hypotension produced by experimental hemorrhage, can be tolerated for considerably longer periods without damage to vital organs since it is not accompanied by compensatory vasoconstriction.[20] This ability of ganglion-blocking agents to reduce blood loss during surgical procedures prompted this attempt to answer the question whether such substances were capable of reducing fluid loss and prolonging survival in animals subjected to an extensive experimental burn. It was also attempted to determine whether animals thus treated were still able to benefit from the survival-promoting qualities of intravenous fluid therapy administered after a considerable postburn delay.

METHODS

Male albino rats, of a local pure strain, ranging in weight from 150 to 265 Gm. were used. The data represent experiments performed on some 450 rats. The animals were housed in wire cages at a room temperature of 23 ± 2° C. and fed a balanced diet in the form of dried pellets and water ad libitum. Burns were produced according to the method described by McCarthy[13] with the following slight modification: anesthesia was induced by placing the animals under a 3-L. bell-jar through which an oxygen-ether mixture was allowed to pass for exactly four minutes. This time was found adequate for producing a type of anesthesia whereby the rats would be awake within one minute postburn. The burn was

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produced by immersing the backs of the anesthetized rats into water at 90 ± 2° C. for exactly thirty-five seconds. Following immersion the excess water was removed by rolling the animals upon a cloth towel and on completion the substances to be tested injected into the lateral tail vein in the following quantities:

1. Azamethonium (Pendiomid*): 2 mg. in 0.2 c.c. of 0.9 per cent NaCl.
2. Hexamethonium bromide (Vegolysen†): 1 and 2 mg. in 0.2 c.c. of 0.9 per cent NaCl per mg.
3. Controls received an equal quantity of 0.9 per cent NaCl.

Time of death was recorded to the nearest five minutes. Pretreated animals received 2 mg. azamethonium in a similar manner one-half hour prior to burning. At death the burned skin was excised, the burned area copied onto cellophane, and the area determined by planimetry. The excised skin was cut into two sections and the natural curvature of the skin straightened by an additional incision at right angles to the dividing cut. This procedure permitted adequate flattening of the skin by hand under the cellophane without additional mechanical stretching. Total body surface was calculated from the formula: $S = KW^{0.6}$, where $K = 12.54$ and $W =$ weight of animal.

Preparation of Animals for Intravenous Infusion.—In order not to add the trauma of an operation to the standard burn procedure rats were anesthetized 24 to 72 hours prior to burning and a P20 polyethylene catheter filled with 0.1 per cent heparin introduced into the priorly exposed right jugular vein. Exposure was obtained by a small (ca. 0.5 cm.) incision on the ventral aspect of the neck immediately above the rib cage. Once introduced into the vein the catheter was passed down into the direction of the right heart chamber until it rested just above or below the opening into the right atrium. The catheter was secured in the vein with silk suture ties and the distal segment of the vein ligated. The free end of the catheter, which had been previously knotted in order to prevent leakage of its contents, was next passed under the skin around to the back of the neck by sliding it through a wide hypodermic needle introduced by a stab incision in back of the occiput. The catheter was thus resurfaced in back of the head and at the point of emergence anchored to the skin. The protruding segment (ca. 2-3 cm. long) was pushed under the skin of the back with a pair of forceps, and the ventral incision completely closed. Following the operation the rats received 20,000 units of penicillin intramuscularly and were placed into individual wire cages with food and water being allowed ad libitum. In the described location behind the neck the catheter is beyond the animal's reach and patency and thus accessibility to intravenous infusion was found as late as one week following insertion. The burning procedure for these animals was identical to the one described above except that the small segment of catheter reburied at the conclusion of the operation was first withdrawn from its subcutaneous position in order to prevent damage by the hot water. Animals operated upon received 2 mg. azamethonium (Pendiomid) into a lateral vein of the tail immediately postburn and the polyethylene segment was joined to a P90 polyethylene catheter which was, in turn, attached to a hypodermic needle and syringe through which fluid was administered. Fluid therapy consisted of 1.4 per cent NaCl amounting to 5 per cent body weight and delivered as a constant infusion from the eighth to the twelfth hour postburn. At the end of this period surviving animals were returned to their cages and sacrificed after forty-eight hours in order to determine the area burned. Location and patency of the jugular catheter were checked at autopsy in every case.

Fisher's t-test[8] for the significance of differences of mean survival times was employed to determine the effect of therapy.

RESULTS

In order to ascertain that differences in mean survival time were due to no other factor(s) than treatment, the 2 groups comprising the upper and

*N, N, N′, N′, –3 pentamethyl-N, N-diethyl-3-aza-pentane-1, 5-diammonium dibromide was generously supplied by Dr. H. J. Bein of Ciba Ltd., Basel, Switzerland.

†Vegolysen made by May & Baker Ltd., Degenham, England.

lower extremes in weight and burned surface were compared. In Fig. 1 the two extreme weight groups, that is, 150 to 190 and 220 to 265 grams, were compared and the difference in mean survival time found not to be significant, p being greater than 0.05. In Fig. 2 the two extremes in burned surfaces, that is, 24 ± 2 per cent and 29 ± 2 per cent, showed no significant difference between means, t equalling 0.378 and p being greater than 0.70. Based on these findings all subsequent data included animals weighing 150 to 265 grams and having received a body burn of 27 ± 4 per cent of their body surface.

The influence of azamethonium (Pendiomid), given either one-half hour prior to or immediately following the burn, on mean survival time is illustrated in Fig. 3. In both instances the difference proved to be significant, t equalling 4.366 and p being less than 0.001 in the pretreated group while t equalled 3.265 and p was less than 0.01 in the case of after-treatment.

The amount of azamethonium employed was in the LD_{50} range for the mouse, a range which may be assumed to apply to the rat as well.[3] This dosage was chosen in order to make evident any difference which might be afforded by treatment. Since both the heuristic and practical values of after-treatment exceed that of pretreatment, the effects of hexamethonium were investigated only when this substance was given in the postburn period. The results are presented in Fig. 4. Thus if hexamethonium is given immediately postburn in a quantity of 1 mg. intravenously mean survival time is significantly prolonged, t being 3.125 and p less than 0.01. Increasing the amount of hexamethonium given to 2 mg. results in a disappearance of the beneficial effect and treated animals die as rapidly as controls, t for the difference of the means being 0.04.

Fig. 5 represents the data obtained on pre-operated rats receiving 2 mg. azamethonium immediately postburn and a constant infusion of 1.4 per cent NaCl amounting to 5 per cent of body weight between the eighth and twelfth hour following burning. Treated animals showed no significant prolongation in mean survival time over controls, t being 0.222. On the other hand operated controls showed a significantly longer mean survival than unoperated controls, t being 2.515 and p less than 0.02. In addition, approximately one-half of the operated animals, whether treated or not, still surviving at the time when the saline solution infusion was started, survived beyond the twenty-four-hour period. None of the 450 animals employed in this experiment, including those whose burned surface did not comply with the required standard, survived beyond 16 hours following burning if no fluid was replaced.

Table I summarizes survival at different periods postburn in those groups in which a significant prolongation of mean survival time over corresponding controls was obtained. Although the number of animals in the hexamethonium-treated and operated controls group is too small to allow for definite conclusions, a definite trend by these animals to survive in greater numbers following an extensive burn can be seen. These differences were shown to be statistically significant (see above). After eight hours approximately one-half of the aforementioned two groups were still alive while among controls only some 3 per cent were still surviving.

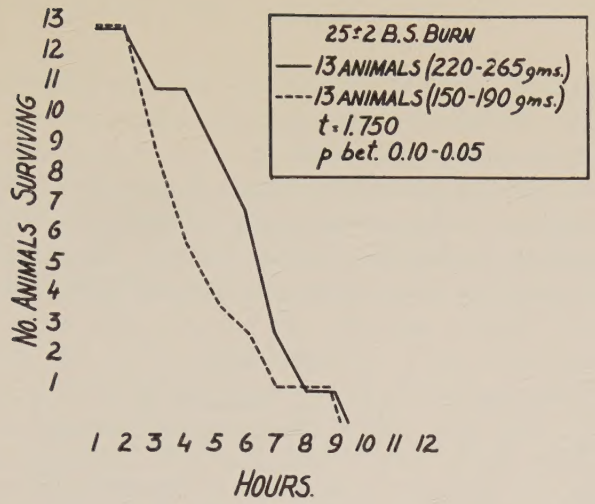

Fig. 1.—Curves comparing survival in two extreme weight groups. Due to small number of animals compared, the number rather than the per cent surviving is plotted on the ordinate.

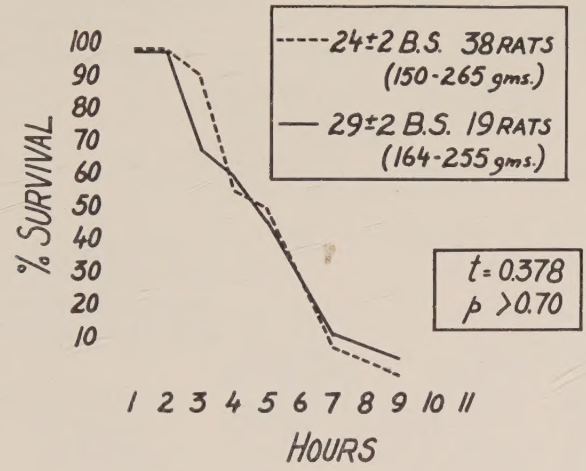

Fig. 2.—Curves comparing survival in two extremes in burned surface area.

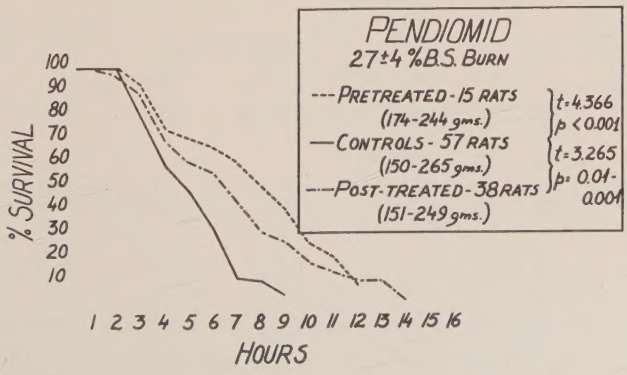

Fig. 3.—Survival curves for rats receiving a standard burn, one group being pretreated and another immediately after burning with 2 mg. azamethonium (Pendiomid) intravenously. Pretreatment one-half hour prior to burning.

Table II presents a general summary of the experiments performed and the data obtained. Statistically significant differences in mean survival time were found when comparison was made in the following groups: (1), Pretreated with 2 mg. azamethonium and corresponding controls; (2), Post-treated with 2 mg. azamethonium and corresponding controls; (3), Post-treated with 1 mg. hexamethonium and corresponding controls; (4), Animals operated twenty-four to seventy-two hours prior to burning and unoperated controls.

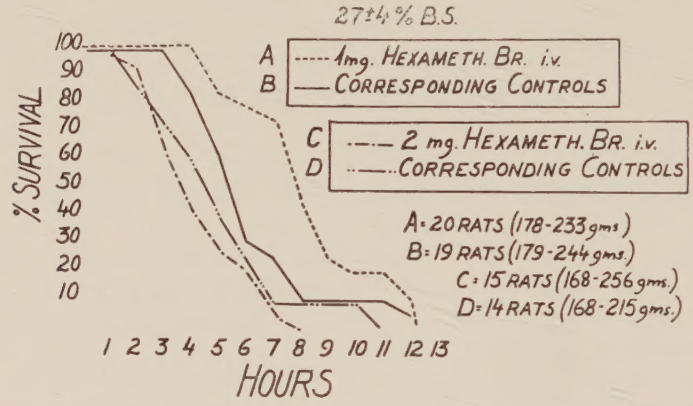

Fig. 4.—Survival curves for burned rats treated with 1 or 2 mg. hexamethonium bromide intravenously immediately postburn.

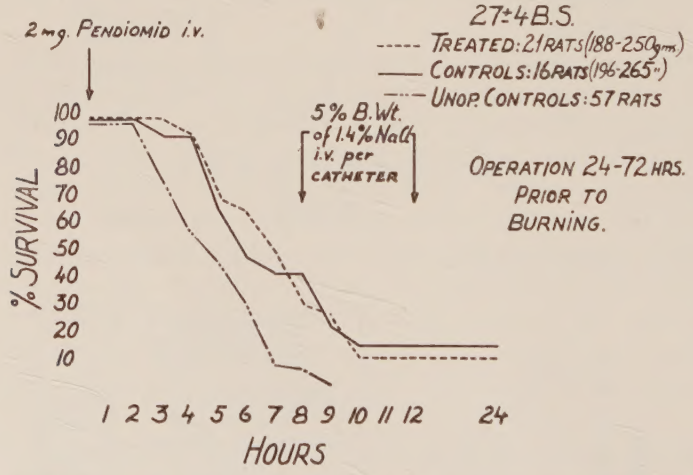

Fig. 5.—Survival curves of preoperated rats receiving 2 mg. azamethonium (Pendiomid) intravenously immediately postburn and an infusion of 1.4 per cent NaCl (5 per cent body weight) from the eighth to the twelfth hour postburn.

DISCUSSION

Controlled hypotension which may be produced by the use of ganglion-blocking agents has found application in surgery partially by virtue of its ability to reduce both bleeding during operations and the frequency of post-

operative complications usually associated with great losses of fluid.[4, 6] Such fluid loss plays a major part in producing the classical picture of shock in the experimental animal and its significance in promoting survival, when properly replaced, has recently been well documented by McCarthy and coworkers[15, 16] in the rat and Millican and associates[17] in the mouse subjected to a standardized burn. Aside from other changes contributing to shock and which it may incite, fluid loss is always accompanied by an increase in peripheral vasoconstrictor tone thus allowing the organism a relatively efficient means of redistributing the residual blood volume. Once this situation has arisen, removal of the tone, be it by anatomic or chemical means, can only prove disastrous since the resulting peripheral pooling will deprive vital organs of a critical supply of blood. The sequence of these events and their importance for survival is demonstrated by studies in which reduction in vasoconstrictor tone has been produced either before or after a standard traumatic procedure.

TABLE I. SURVIVAL AT DIFFERENT PERIODS POSTBURN*

TREATMENT	PER CENT AREA BURNED	NO. RATS	ALIVE AT 2 HR.	4 HR.	6 HR.	8 HR.	10 HR.	12 HR.
Controls	27 ± 4	76	75	49	24	2	2	0
Pendiomid	27 ± 4	38	36	26	21	11	7	4
Hexamethonium	27 ± 4	20	20	20	16	9	4	2
Operated controls	27 ± 4	16	16	15	8	7	3	3

*Indicates the number of surviving animals in various groups at different periods following burning. Included are only those groups which show a statistically significant prolongation of mean survival time over controls.

TABLE II. SUMMARY OF THE EXPERIMENTS AND DATA OBTAINED

GROUPS COMPARED	NO. RATS	PER CENT AREA BURNED	WEIGHT (GM.)	MEAN* SURVIVAL TIME ± S. E.	t	p
150-190 gm.	13	25 ± 2	—	265 ± 34	1.750	0.10-0.05
220-265 gm.	13	25 ± 2	—	349 ± 33		
24 ± 2 B.S.	38	—	150-265	280 ± 23	0.378	>0.70
29 ± 2 B.S.	19	—	164-255	294 ± 24		
Pretreated with 2 mg. Pendiomid	15	27 ± 4	172-244	469 ± 54	4.366	<0.001
Controls	57	27 ± 4	150-265	290 ± 16		
Post-treated with 2 mg. Pendiomid	38	27 ± 4	151-249	401 ± 34	3.265	0.01-0.001
Post-treated with hexamethonium (1 mg.)	20	27 ± 4	178-233	481 ± 33	3.125	0.01-0.001
Controls	19	27 ± 4	179-249	356 ± 29		
Post-treated with hexamethonium (2 mg.)	15	27 ± 4	168-256	276 ± 37	0.04	—
Controls	20	27 ± 4	168-227	278 ± 31		
Operated + Pendiomid	21	27 ± 4	188-250	381 ± 22	0.222	—
Operated controls	16	27 ± 4	196-265	373 ± 29	2.515	0.02-0.01
Unoperated controls	57	27 ± 4	150-265	290 ± 16		

*Time in minutes.

Freeman[9] demonstrated originally that totally sympathectomized dogs withstood hemorrhagic hypotension better than corresponding unoperated controls although they reacted with greater changes in blood pressure to smaller losses of blood. Similar results have been obtained more recently by Baez, Zweifach, and Shorr[2] who produced a form of peripheral sympathectomy by pretreating their animals with Dibenamine and its congeners.[22] Glasser and Page[10] subjected dogs treated with tetraethylammonium to a standard hemorrhage and found improved survival in both the good and bad prognosis groups. Significant is the fact that in all studies where chemical blockade was attempted in the presence of hemorrhagic hypotension, improved survival was found only when blocking had been carried out prior to bleeding. Glasser and Page treated dogs also after hemorrhage had taken place, but analysis of their results is complicated by the fact that these animals were allowed to redraw lost blood from the reservoir in order to maintain a prescribed blood pressure level which had been excessively lowered by treatment with the ganglion-blocking agent.

Hypotension alone, in the absence of fluid loss, is not as deleterious as hypotension produced by hemorrhage. This is due to the prolonged compensatory vasoconstriction which invariably follows the latter. Aynlan and associates[1] found that dogs subjected to chemical hypotension with Arfonad, a ganglion-blocking agent, exhibited a lower mortality than dogs in which hypotension had been produced by hemorrhage. Although the hemodynamic readjustments to fluid replacement in an animal subjected to an extensive burn or hemorrhage are quantitatively similar, these two shock-producing methods differ in one important aspect, namely, the rate of initial fluid loss. In laboratory hemorrhage this rate can be well controlled by the individual experimenter and rapidity of bleeding is very much a matter of choice. In a burn, however, certain parameters (blood pressure, depth of burn) control the rate of loss and these combine in such manner that maximum loss is achieved much later than after hemorrhage.[5, 19] The difference in this rate of fluid loss is important because it allows for an explanation of the beneficial effect afforded by ganglion-blocking agents when administered immediately following the burn. The resulting hypotension is achieved by removing excessive vasoconstrictor tone arising in the early stages postburn as a result of (1) an increased discharge of nociceptive stimuli from the burned area and (2) fluid loss into the affected region. Removal of excessive tone occurs at a moment when adjustment to such a change is still possible, that is, when the animal is still in possession of a near normal effective extracellular fluid volume. Similar conclusions have already been proposed by Phemister and Laestar[21] who found that spinal anesthesia protected dogs against shock developing subsequent to limb trauma. In regard to the added pressor effect of nociceptive stimuli, Wang and Overman[24] found that dogs submitted to hemorrhage alone survived the period of shock better than dogs in which artificial stimulation of afferent nerves had been added to hemorrhage or muscle trauma as the shock-producing method.

As blood pressures were not recorded in our experiments it is not possible to substantiate directly the above statements. Repeated measurement of

plasma and red-cell volume with and without treatment may provide the full answer; however, on the basis of the known actions of ganglion-blocking agents in man and the experimental animal the explanation appears plausible.[4, 20] Chemical hypotension probably reduces the rate at which fluid is lost into the burned area by reducing blood pressure and prolongs mean survival time by delaying the point beyond which additional loss is no longer compatible with life.

The finding that animals previously operated survive longer than unoperated controls, whether the former have been treated or not, makes such a simplified explanation difficult to accept. Nevertheless, in the absence of evidence to the contrary, it is very likely that we are faced with two different phenomena and the survival value of conditioning to trauma is probably greater than that of hypotension produced with the intention of reducing fluid loss. Conditioning to trauma has recently found renewed support in rats subjected to other forms of experimental injury,[23] but the mechanism responsible has not received satisfactory explanation. It has been suggested that the liver might play a major part in giving rise to this phenomenon.[7, 23] Our experiments provide no insight into the pathways by which this phenomenon is mediated but it is apparent that if hypotension does occur in the operated and treated animals then it has no great positive influence on survival when compared to corresponding controls. Thus ganglion-blocking agents, when used in the immediate postburn period, have, in all probability, primarily a palliative effect and little influence on survival beyond a certain point if fluid therapy is completely disregarded.

Another possible explanation for the beneficial effect of substances exerting lytic or blocking effects on the autonomic nervous system is provided by the recent finding by Henry[12] that hyperkalemia due to excessive epinephrine release following a burn may be prevented by treating animals with piperoxan hydrochloride (Benodaine hydrochloride).

Interesting to note is the fact that the intravenous infusion of slightly hypertonic saline solution (1.4 per cent), administered in amounts of 5 per cent of body weight eight hours postburn was able to promote survival beyond the twenty-four-hour period. As mentioned earlier none of the animals who did not receive replacement therapy survived longer than sixteen hours. According to McCarthy and co-workers[15, 16] the quantities of saline solution used are inadequate when ten-day survival is used as a criterion. However, it is clear that even these supposedly inadequate quantities allowed half of the animals receiving such therapy to survive beyond the period which lack of fluid replacement would have allowed. Whether this is also due to the conditioning phenomenon is impossible to state at the moment.

CONCLUSIONS

1. Treatment of rats subjected to a standard hot water burn at 90 ± 2° C. for thirty-five seconds with ganglion-blocking agents (hexamethonium or azamethonium, a substituted pentamethonium compound) has a positive, statistically significant effect in prolonging mean survival time.

2. In the absence of other direct evidence it is suggested that such treatment in the immediate postburn period probably results in hypotension which in turn is responsible for a reduction in the rate of fluid lost into the burned area.

3. Body trauma in the form of an operation performed recently prior to burning increases the animal's resistance to burning to the extent that it survives longer than the unoperated control. The use of blocking agents in conditioned animals affords no additional protection. This has been found to be the case in animals operated upon twenty-four to seventy-two hours prior to burning.

4. Animals that show evidence of conditioning and have received an extensive body burn are still able to benefit from the survival-promoting qualities of intravenous hypertonic (1.4 per cent) saline when the latter is given as late as eight hours postburn.

REFERENCES

1. Aynlan, W. G., Shingleton, W. W., Benson, W. R., Stephen, R. C., Salem, M. and Taylor, H. M.: A Study of Liver Damage Following Induced Hypotension, SURGERY **36**: 375, 1954.
2. Baez, S., Zweifach, B. W., and Shorr, E.: Protective Action of Dibenamine Against the Fatal Outcome of Hemorrhagic and Traumatic Shock in Rats, Fed. Proc. **11**: 7, 1952.
3. Bein, H. J.: Personal communication.
4. Bein, H. J., and Meier, R.: Zur Frage der Shockbekämpfung mit Pendiomid, Anaesthesist 3: 25, 1954.
5. Cope, O., and Moore, F. A.: The Redistribution of Body Water and the Fluid Therapy of the Burned Patient, Ann. Surg. **126**: 1010, 1947.
6. Enderby, G. E. H.: Controlled Circulation With Hypotensive Drugs and Posture to Reduce Bleeding in Surgery; Preliminary Results With Pentamethodium Iodide, Lancet **1**: 1145, 1950.
7. Fine, J.: The Bacterial Factor in Shock. Springfield, Ill., 1954, Charles C Thomas, Publisher.
8. Fisher, R. A.: Statistical Methods for Research Workers, ed. 12 Edinburgh, 1954, Oliver & Boyd, Ltd.
9. Freeman, N. E., Shaffer, S. A., Schechter, A. E., and Holling, H. E.: Effect of Total Sympathectomy on Occurrence of Shock from Hemorrhage, J. Clin. Invest. **17**: 359, 1938.
10. Glasser, O., and Page, I. H.: Experimental Hemorrhagic Shock; A Study of its Production and Treatment, Am. J. Physiol. **154**: 297, 1948.
11. Greene, L. C., Stuart, E. G., and Joralemon, J.: Survival Study of Thermally Injured Rats Treated with Piromen, Proc. Soc. Exper. Biol. & Med. **82**: 39, 1953.
12. Henry, C. L.: The Role of Epinephrine in Hyperkalemia of Acute Experimental Burns, Proc. Surg. Forum, Clin. Congress Am. Coll. Surgeons, 1954, Philadelphia, 1955, W. B. Saunders Company.
13. McCarthy, M. D.: A Standardized Back Burn Procedure for the White Rat Suitable for the Study of the Effects of Therapeutic and Toxic Agents on Long-Term Survival. J. Lab & Clin. Med. **30**: 1027, 1945.
14. McCarthy, M. D., and Blackburn, V.: Effect of Piromen on Survival Following Severe Thermal Injury in Rats, Science **119**: 348, 1954.
15. McCarthy, M. D., and Draheim, J.: Survival of Thermally Injured Rats Infused with Saline, Polyvinylpyrrolidone, Dextran, and Oxypolygelatine. Proc. Soc. Exper. Biol. & Med. **79**: 346, 1952.
16. McCarthy, M. D., and Newlin, N.: The Range of Efficacy of Sodium Chloride Solutions in Treating Severe Thermal Injury in the Rat, J. Lab. & Clin. Med. **41**: 416, 1953.
17. Millican, C. R., Tabor, H., and Rosenthal, S. M.: Traumatic Shock in Mice. Comparison of Survival Rates Following Therapy, Am. J. Physiol. **170**: 179, 1952.
18. Moyer, C. A.: An Assessment of the Therapy of Burns; A Clinical Study, Ann. Surg. **137**: 628, 1953.

19. Noble, R. P., and Gregersen, M. I.: Blood Volume in Clinical Shock. II. The Extent and Cause of Blood Volume Reduction in Traumatic, Hemorrhagic and Burn Shock, J. Clin. Invest. **25**: 172, 1946.
20. Paton, W. D. M.: Ganglionic Blocking Agents. With Special Reference to the Effect of Hexamethonium on the Cardiovascular System, Brit. M. Bull **8**: 310, 1952.
21. Phemister, D. B., and Laestar, C. H.: Local Fluid Loss, Nerve Stimuli and Toxins in Causation of Shock, Ann. Surg. **121**: 803, 1945.
22. Shorr, E.: in Metabolic Aspects of Shock, Transactions of the Second Conference on Shock, Josiah Macy, Jr., Foundation, New York, 1952.
23. Shorr, E., Baez, S., Metz, D. B., and Zweifach, B. W.: Cross-Resistance to Hemorrhagic Shock in Rats Made Resistant to Drum Trauma; Relation to Hepatic VDM Inactivation Mechanism, Fed. Proc. **11**: 147, 1952.
24. Wang, S. C., and Overman, R. R.: Neurogenic Factor in Experimental Shock: Summary of Recent Studies Including Observations on Procainized and Spinal Dogs. Ann Surg. **129**: 207, 1949.

CURRICULUM VITAE

Ich, Myron B. Laver, Bürger der Vereinigten Staaten von Amerika, wurde am 17. August 1926 in Bukarest, Rumänien, geboren.
1941 übersiedelte ich nach Amerika, wo ich in New York in das Gymnasium (Forest Hills High School) eintrat. 1943 bestand ich an dieser Schule die Matura mit Erfolg. Im Frühling 1943 begann ich meine Studien im Earlham College, Richmond, Indiana, die ich jedoch im Winter 1944 infolge Militärdienst bis und mit Herbst 1946 unterbrechen musste. Aus dem Militärdienst zurückgekehrt, setzte ich meine Studien am Earlham College fort und schloss sie 1948 erfolgreich mit dem „Bachelor of Arts" Diplom ab.
Von 1948 bis 1950 erweiterte ich meine Studien in Zoologie an der Universität von Chicago und begab mich anschliessend in die Schweiz, um an der Universität von Bern Medizin zu studieren. Im Frühling 1952 bestand ich mit Erfolg das zweite propädeutische Examen, setzte anschliessend meine Studien an der Universität Basel fort und promovierte am 29. Mai 1956.